Communications to the editor

NEW ANTITUMOR ANTIBIOTICS, DITRISARUBICINS A, B AND C

Sir:

During the screening for new antitumor antibiotics, new members of the anthracycline group were discovered in culture broths of a strain designated as MG344-hF49 and classified as *Streptomyces cyaneus*, and they were named ditrisarubicins A, B and C. These antibiotics inhibit the growth of Gram-positive bacteria and prolong the survival period of mice bearing leukemia L-1210. Each ditrisarubicin consists of β-rhodomycinone[1], two molecules of rhodosamine[2] and two to four other hexoses.

The strain MG344-hF49 was cultured at 27°C for 72 hours on a rotary shaker in a 500-ml Erlenmeyer flask containing 110 ml medium of the following composition: 2% galactose, 2% dextrin, 1% soybean meal ("Bacto-soytone", Difco Laboratories), 0.5% corn steep liquor and 0.1% $CaCO_3$, pH 7.0 before sterilization. The cultured broth thus prepared in 3 flasks was used to inoculate 15 liters of the same medium in a 30-liter jar fermentor, and the fermentation carried out at 27°C for 6 days under agitation at 300 rpm and aeration at 15 liters per minute. The production of the antibiotics was assayed by a cylinder plate method testing for activity against *Bacillus subtilis* PCI219. Ditrisarubicins A, B and C were separated by a silicic acid thin-layer chromatography (Kieselgel $60F_{254}$, Merck Co.). Their Rf values determined in several solvent systems are shown in Table 1.

The culture broth (13 liters) was adjusted to pH 4.5 and filtered. The glycosides in the filtrate were extracted with butyl acetate (10 liters) at pH 8.0, and the active extract was concentrated to dryness. The dried residue thus obtained was dissolved in a small amount of chloroform, and subjected to a silicic acid column chromatography. After washing with chloroform, the active fraction was eluted with chloroform - methanol (100: 2) and concentrated *in vacuo*. The crude dark red powder (115 mg) thus obtained was spotted onto silicic acid thin-layer plates and developed with chloroform - methanol - aqueous ammonia (100: 5: 0.05). The bands assigned to ditrisarubicins A, B and C were scrapped off, and the antibiotics eluted with chloroform - methanol (10: 1), respectively. After drying, each ditrisarubicin was subjected to a Sephadex LH-20 column chromatography developed with methanol. Thus, 8.5 mg of ditrisarubicin A, 15 mg of ditrisarubicin B and 5.0 mg of ditrisarubicin C were obtained in pure forms.

Table 1. Rf values* of ditrisarubicins A, B and C.

Ditrisarubicins	Solvent systems		
	(1)	(2)	(3)
A	0.63	0.42	0.09
B	0.79	0.56	0.32
C	0.65	0.47	0.11

Solvent systems:
(1) Chloroform - methanol - acetic acid, 20: 2: 0.1.
(2) Chloroform - methanol - aqueous ammonia, 100: 5: 0.02.
(3) Ethyl acetate - methanol, 10: 1.
* On Kieselgel $60F_{254}$ SiO_2 (Merck Co.).

The physicochemical properties of ditrisarubicins A, B and C are as follows:

Ditrisarubicin A: mp 184~187°C (decomp.); $[\alpha]_D^{25}$ +119° (*c* 0.1, $CHCl_3$); λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 234 (320), 252 (200), 290 (58), 496 (100), 530 (81), 588 (42); $\lambda_{max}^{0.1N\ HCl\text{-}90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 234 (341), 253 (187), 290 (66), 495 (121), 527 (77); $\lambda_{max}^{0.1N\ NaOH\text{-}90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 238 (385), 288 (92), 567 (150), 602 (133); IR (KBr) cm^{-1} 3450, 2950, 2770, 1740, 1605, 1470, 1450, 1405, 1380, 1300, 1240, 1200, 1170, 1120, 1100, 1020, 970, 820. *Anal.* Calcd. for $C_{60}H_{82}N_2O_{22}$: C 60.90, H 6.99, N 2.37, O 29.75. Found: C 60.15, H 7.28, N 2.41, O 30.16. FD-MS: *m/z* 1,183 $(M+1)^+$.

Ditrisarubicin B: mp 196~198°C (decomp.); $[\alpha]_D^{25}$ +132° (*c* 0.1, $CHCl_3$); λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 234 (320), 252 (201), 290 (58), 496 (100), 530 (81), 588 (42); $\lambda_{max}^{0.1N\ HCl\text{-}90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 234 (341), 253 (188), 290 (65), 495 (120), 527 (75); $\lambda_{max}^{0.1N\ NaOH\text{-}90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 238 (383), 288 (90), 567 (147), 602 (130); IR (KBr) cm^{-1} 3450, 2950, 2770, 1740, 1605, 1470, 1450, 1410, 1305, 1245, 1200, 1170, 1120, 1100, 1050, 1020, 970, 930, 830. *Anal.* Calcd. for $C_{60}H_{80}N_2O_{22}$: C 61.01, H 6.83,

N 2.37, O 29.80. Found: C 60.02, H 7.01, N 2.30, O 30.67. FD-MS: m/z 1,181 $(M+1)^+$.

Ditrisarubicin C: mp 173~176°C (decomp.); $[\alpha]_D^{25}$ +167° (c 0.1, $CHCl_3$); λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$) 233 (330), 253 (178), 290 (58), 495 (108), 528 (78), 585 (35); $\lambda_{max}^{0.1N\ HCl\text{-}90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 234 (347), 254 (183), 290 (62), 495 (121), 528 (75); $\lambda_{max}^{0.1N\ NaOH\text{-}90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$) 241 (348), 300 (75), 568 (134), 607 (119); IR (KBr) cm^{-1} 3450, 2950, 2770, 1740, 1700, 1605, 1470, 1450, 1405, 1300, 1245, 1200, 1120, 1005, 970, 820. *Anal.* Calcd. for $C_{60}H_{80}N_2O_{21}$: C 61.84, H 6.92, N 2.40, O 28.83. Found: C 61.01, H 7.15, N 2.35, O 29.54. FD-MS: m/z 1,165 $(M+1)^+$.

Acid hydrolysis of ditrisarubicins in 0.1 N HCl for 30 minutes at 85°C gave β-rhodomycinone, which was extracted with chloroform and identified by direct comparison with an authentic sample in a silicic acid thin-layer chromatography. The identity was also supported by mass (m/z 386 $(M)^+$) and ^1H NMR spectra[1]. The sugar moieties which were present in the aqueous phase of the hydrolysate were subjected to a silicic acid thin-layer chromatography developed with 1-butanol - acetic acid - water (4: 1: 1). Different sets of hexoses were obtained from each ditrisarubicin and those shown in Table 2 were identified by comparison with authentic samples prepared from aclacinomycins A, B and N[3]. The number of molecules of these hexoses in each ditrisarubicin was shown by mass spectroscopy (Table 2).

Hydrogenolysis of ditrisarubicins A, B or C with palladium catalyst in methanol at room temperature for 30 minutes yielded in all cases the same trisaccharide in addition to the γ-rhodomycinone trisaccharides, A′, B′ or C′, respectively (Fig. 1). Further hydrolysis of A′, B′ and C′ in 0.1 N HCl for 30 minutes at 85°C gave γ-rhodomycinone[4] in addition to three different hexoses. The molecular weights determined by field desorption mass spectroscopy of A′, B′ and C′ and their hexose components are shown in Table 3. Thus, we propose the structures shown in

Table 2. Hexose components of ditrisarubicins A, B and C.

Hexose	Molar ratio		
	A	B	C
Rhodosamine	2	2	2
2-Deoxyfucose	2	2	1
Rhodinose	0	0	1
Cinerulose A	1	0	0
Cinerulose B	1	2	1
Aculose	0	0	1*

* Aculose was identified by ^1H NMR spectrum.

Table 3. Hexose components and molecular weights of A′, B′ and C′ (γ-rhodomycinone glycosides).

Compound	MW m/z $(M+1)^+$	Hexose components
A′	770	RhN, deFuc, CinA
B′	768	RhN, deFuc, CinB
C′	754	RhN, Rho, CinA

RhN: Rhodosamine, deFuc: 2-deoxyfucose, Rho: rhodinose , CinA: cinerulose A, CinB: cinerulose B.

Fig. 1. Hydrogenolysis of ditrisarubicin A.
R_1 and R_2 are I and II, represented in Fig. 2, respectively.

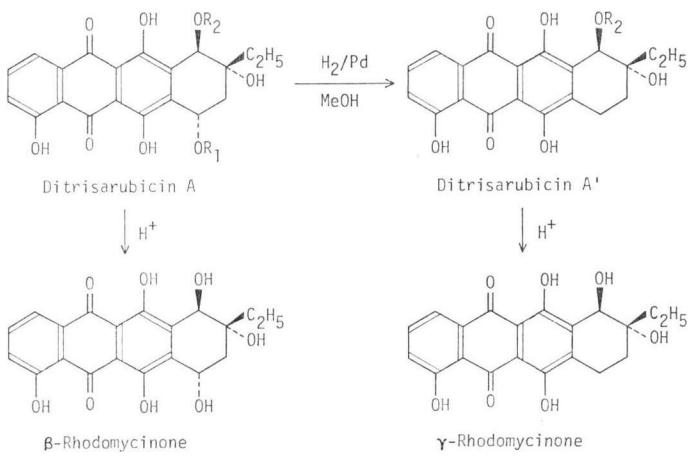
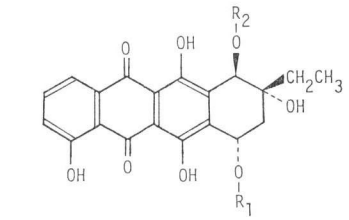


Fig. 2. Structures of ditrisarubicins A, B and C.



	R_1	R_2
Ditrisarubicin A	I	II
Ditrisarubicin B	I	I
Ditrisarubicin C	I	III

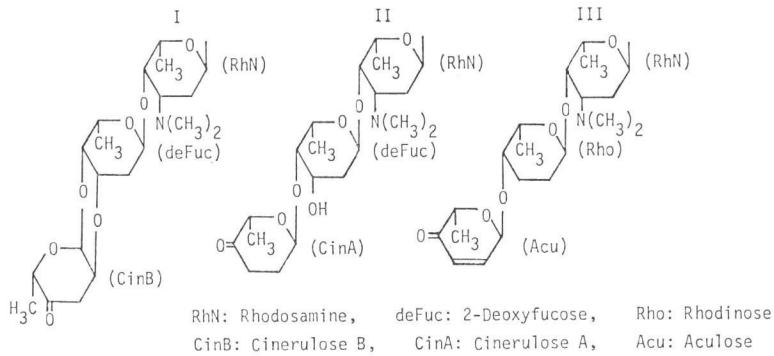


Table 4. Minimum inhibitory concentrations of ditrisarubicins A, B and C.

Test organism	MIC (μg/ml)		
	A	B	C
Staphylococcus aureus FDA 209P	<0.2	<0.2	<0.2
S. aureus Smith	0.39	0.78	0.39
S. aureus MS8710	<0.2	0.39	<0.2
S. aureus MS9610	0.39	0.39	<0.2
Micrococcus lysodeikticus IFO3333	<0.2	<0.2	<0.2
Bacillus subtilis PCI219	<0.2	0.39	0.39
B. cereus ATCC10702	0.39	0.39	0.39
Corynebacterium bovis 1810	0.78	<0.2	<0.2
Escherichia coli NIHJ	50	>50	>50
Klebsiella pneumoniae PCI602	50	50	>50
Salmonella typhi T-63	50	>50	>50
Serratia marcescens	50	50	50
Proteus vulgaris OX19	50	50	50
Pseudomonas aeruginosa A3	12.5	25	25

Fig. 2 for ditrisarubicins A, B and C. Detailed structural studies will be reported elsewhere.

Among known β-rhodomycinone glycosides, only β-rhodomycin S2 has six hexoses: it contains rhodinosyl-2-deoxyfucosyl-rhodosaminyl group at C-7 and rhodinosyl-rhodinosyl-rhodosaminyl group at C-10[5]. The sugar components of β-rhodomycin S2 are different from those of ditrisarubicins. Thus, ditrisarubicins A, B and C were confirmed to be new members of anthracycline antibiotics.

The antimicrobial activity of ditrisarubicins A, B and C was examined by agar dilution method

Table 5. Antitumor activity of ditrisarubicins A, B and C against L-1210 leukemia.

Dose (mg/kg/day)	T/C (%)		
	A	B	C
0.25	91	108	114
0.125	119	159	148
0.0625	148	148	142
0.0313	148	136	182
0.0157	136	114	142

Inoculum: L-1210 cells, 10^5 cells/CDF_1 mouse, i.p.
Treatment: Day 0~10, i.p.
Prolongation rate (T/C, %)=mean survival period of mice treated/mean survival period of the control.

Table 6. Effect of ditrisarubicins A, B and C on the growth and macromolecular synthesis of cultured L-1210 leukemia cells.

Compound	IC_{50} (μg/ml)			Ratio $\frac{IC_{50}\ DNA}{IC_{50}\ RNA}$
	Growth	DNA synthesis	RNA synthesis	
Ditrisarubicin A	0.0005	0.31	0.023	13.5
〃 B	0.0011	0.29	0.029	10.0
〃 C	0.0007	0.14	0.014	10.0

IC_{50} values were estimated by Probit analysis.
Cytotoxicity was determined on day 2 culture.

and the results are shown in Table 4. As shown in Table 5, ditrisarubicins prolonged the survival period of CDF_1 mice to which L-1210 leukemia cells were intraperitoneally inoculated. Ditrisarubicins A, B and C showed marked cytotoxicity against cultured L-1210 leukemia cells and inhibited preferentially RNA synthesis, as shown in Table 6.[6] LD_{50} in mice was 5~10 mg/kg by the intraperitoneal injection.

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